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H_2S—A Settlement Cue or a Toxic Substance For *Capitella* sp. I Larvae?

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Abstract. In small-scale laboratory experiments, organic-rich sediment lacking sulphide elicited settlement and metamorphosis in freshly hatched *Capitella* sp. I larvae, so that 90% settled within 30 min after hatching. Settlement times of somewhat older larvae were even shorter; 90% settled into the mud in less than 5 min. The addition of sulphide to these treatments (0.5 m*M*, 1.0 m*M*, and 2.0 m*M*) delayed settlement, so that it took several hours for 90% of the larvae to settle. Many of these larvae showed abnormal behavior and settled distant from the sediment. Sulphide alone (without sediment) enhanced settlement in a concentration-dependent manner (0.5–2.0 m*M*), as previously reported by Cuomo (1985). However, this response occurred over 12–24 h and abnormal larval settling behavior was observed. Hypoxia produced a similar response.

Considerations of behavior and swimming capabilities of *Capitella* larvae, near-bottom hydrodynamic conditions in the field, and the time course of these responses to organic-rich sediment, sulphide, and hypoxia, lead to the conclusion that sulphide is not a settlement cue promoting habitat selection in *Capitella* sp. I larvae. The apparent enhancement of settlement by sulphide is hypothesized to be a sub-lethal toxic effect.

Introduction

The opportunistic polychaete, *Capitella capitata*, is regularly found in sediments of high organic content (Pearson and Rosenberg, 1978), where bacterial decomposition can lead to high concentrations of H_2S. Cuomo (1985) described H_2S as a settlement cue for *Capitella* sp. I (as determined by Grassle and Grassle, 1976) larvae. This was the first time that H_2S was shown to induce a larval response, and Cuomo hypothesized that this cue might explain the high abundances of *Capitella* species in sulphide-rich sediments.

However, there may be an alternative explanation for the effects of H_2S on the settlement of *Capitella* sp. I larvae. The toxicity of H_2S is well documented (National Research Council, 1979) but the effects are reversible when concentrations diminish (Degn and Kristensen, 1981; Torrans and Clemens, 1982). Sub-lethal concentrations of H_2S could trigger larval settlement and/or metamorphosis by physiologically 'shocking' the larvae. Then, as concentrations diminish, recovery might occur with subsequent normal development. Since H_2S is toxic to most living organisms, it would probably 'shock' other larvae besides *Capitella* thereby enhancing settlement of many different species. Other explanations for the high abundances of *Capitella* in H_2S-rich sediments, *e.g.*, differences in survival of larvae and adults, would seem more likely.

To test the proposed 'toxicity' hypothesis, *in vitro* experiments similar to those of Cuomo (1985) were performed. *Capitella* sp. I larvae were exposed to different H_2S concentrations in petri dishes for 24 h. Care was taken to observe behavior throughout the entire settlement process. Additional experiments were conducted to investigate (a) behavior in the presence of a natural substrate by introducing sediment and then testing settlement time with and without H_2S, (b) how long larvae take to settle after hatching by observing settlement behavior of freshly hatched larvae, and (c) the specificity of the settlement response to H_2S by inhibiting the same metabolic pathways as H_2S, namely with hypoxia.

Materials and Methods

In this paper the term "settlement" is defined as termination of pelagic larval existence and denotes a behavioral response (Scheltema, 1974). "Metamorphosis" is a

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morphological term and describes a developmental process (Burke, 1983). In experiments with sediment, settlement time was noted when larvae entered the mud. In experiments without sediment, settlement as defined above did not occur. To compare results, I designated cessation of effective swimming after loss of prototrochal and telotrochal cilia as settlement. However, as shown in the Discussion, I argue that this process is better defined as metamorphosis.

Capitella sp. I larvae were used in all experiments. This species is one of the most opportunistic of the sibling species complex described by Grassle and Grassle (1976) (previously determined as one single species *Capitella capitata* Fabricius). Larvae were obtained from mass cultures reared at ambient seawater temperatures in the laboratory of Dr. J. P. Grassle (Marine Biological Laboratory, Woods Hole). Worms with brood tubes containing developing embryos were isolated in individual dishes without sediment at 20°C and checked daily for hatching of larvae. All experiments were done at room temperature (21–23°C) in 55 mm diameter glass petri dishes.

Experimental sediment consisted of azoic, organic-rich mud from Sippewissett marsh (MA) that had been passed through a 1 mm mesh sieve, frozen, thawed, and aerated twice. Adult worms were cultured in similar sediment but the mud used in experiments was freshly thawed and had not been used for cultures. Sulphide was not detected in the mud with the colorimetric technique used (minimum detectable conc.: 70 μM) or identifiable by odor (perception threshold: approx. 0.1 μM; Dando *et al.*, 1985).

Settlement behavior of freshly hatched larvae

Larvae were tested for settlement ability immediately after hatching. Brood tubes were examined daily until hatching seemed imminent. Early embryos, visible through the wall of the brood tube, are opaque and filled with whitish yolk, and lack any distinct morphological features. Through the 5–6 days of development at 20°C they gradually change, gaining the features of the fully developed metatrochophore larva. The yolk disappears and the gut takes on a greenish blue cast. Shortly before hatching they start to move within the tube. From this point on, the tube was watched continually until larvae began hatching. All the larvae in a brood tube did not hatch simultaneously; hatching took up to an hour with sometimes one larva, and sometimes several larvae at once crawling and swimming out of the brood tube. This behavior made it impossible to record individual settlement times and therefore brood tubes were opened manually, causing all larvae to hatch simultaneously.

As soon as the first 'naturally' hatching larvae were observed, the brood tube was quickly placed in a dish filled with 30 ml of 0.2 μM filtered seawater. Experimental azoic sediment was placed in a small clump in the middle of the dish and the tube torn apart with two dissecting needles. Behavior was examined with a dissecting microscope and two fiber-optic ring lights placed at opposite sides of the dish until all larvae had settled.

H_2S and hypoxia experiments

The behavior and settlement of *Capitella* sp. I larvae were observed under the conditions listed in Table I. Experiments were designed to investigate sub-lethal toxic effects of H_2S. Since Cuomo (1985) found 10 mM sulphide to be toxic to most *Capitella* sp. I larvae, concentrations between 0.1 and 2.0 mM sulphide were tested. In the field, sulphide concentrations can vary greatly depending on the measurement method used, the type of sediment, the time of year, the depth at which the sample was taken, etc. In the same New England salt marsh sulphide concentrations in the sediment ranged from as low as 1.2 μM (Howarth *et al.*, 1983) to as high as 6 mM (Howes *et al.*, 1985).

Most experiments were run in parallel, *i.e.*, each treatment was tested at least once on a given day with sibling larvae from a single brood. Time was a limiting factor because each experiment required at least one hour of constant observation before the next could be started. Larvae were no older than 24 h post-hatching at the beginning of each experimental day. Since up to nine different treatments were tested on one brood, larvae used at the end of the day were, in some cases, at least 9 h older than those used in the morning. Thus, care was taken to change the sequence in which treatments were tested.

H_2S experiments: H_2S and pH determinations. All sulphide measurements were made colorimetrically using a modified version of the Gilboa-Garber method (Howarth *et al.*, 1983). The desired sulphide concentrations were achieved by adding the needed amount of 100 mM sulphide stock solution. Stock solutions were made by dissolving $Na_2S \cdot 9H_2O$ crystals in deoxygenated, deionized water.

The sulphide concentrations measured just before the larvae were added are shown in Table II. The decreases in sulphide after 1 h, measured in some experiments, are shown in Table III.

All pH measurements were made with an electrode. Deoxygenation through N_2 bubbling and addition of H_2S caused high pH values (up to 9.5) in the dishes. The pH was always adjusted to that of untreated seawater (approx. 8.0) by adding 2.0% HCl. Actual pH values varied between 7.6 and 8.1; the average pH in all experiments ($n = 89$) was 7.97 ± 0.13.

H_2S experiments: H_2S without sediment. Thirty ml of deoxygenated, 0.2 μm filtered seawater were poured into

Table I

Experimental conditions

	H_2S									Hypoxia	
	With sediment				Without sediment						
Sulphide conc. (m*M*)	0*	0.5	1.0	2.0	0*	0.1	0.5	1.0	2.0	$+O_2$ *	$-O_2$
# of replicates	30	7	16	6	7	4	6	7	6	6	6

* = Controls.

a petri dish and the needed amount of 100 m*M* sulphide stock solution added to reach the concentrations listed in Table I. The pH was measured and adjusted to that of untreated seawater. After measuring the sulphide concentration, five larvae were pipetted into the petri dish and the dish was covered with a watch glass. Only larvae from the same brood were used in each experiment. Behavior and settlement were observed continually throughout the first hour using a dissecting microscope with two fiber-optic ring lights on opposite sides of the petri dish as a light source. Dishes were examined every 15 min for the following 2 h and every 30 min thereafter for a total of 24 h. Controls were treated as above, except that no H_2S was added.

H_2S experiments: H_2S with sediment. At the beginning of each experiment a small clump of experimental sediment was placed in the middle of a petri dish. Thirty ml of deoxygenated, 0.2 μm filtered seawater were added carefully so that the mud remained in the center. The same procedure was followed as described above (under *H_2S without sediment*). Larval settlement and behavior were observed continuously for the first hour, every 5 min for the second hour, and every 10 min thereafter until all larvae had settled.

Hypoxia experiments. The effect of reduced oxygen concentrations on larval settlement behavior was tested under conditions similar to the H_2S treatments without sediment. Five or ten larvae (all from one brood) were pipetted into a dish which was then carefully filled to the brim (approx. 35 ml) with 0.2 μm filtered seawater that had been bubbled with N_2 for at least 30 min. The dish was quickly covered with a watch glass of the same size, convex side down, so that there were no bubbles. The rim of the dish was wrapped with adhesive tape. Behavior and settlement were observed for the same time periods and under the same conditions as the H_2S experiments. Controls were treated in the same manner but dishes were not wrapped with tape.

Results

Settlement behavior of freshly hatched larvae

Settlement time and behavior were similar in all seven broods observed. Fifty percent of all larvae tested settled into the sediment in less than 5.5 min (Fig. 1) and in 5 broods the remaining 50% settled within 13–17 min. In two broods settlement times of approx. 30% of the larvae were somewhat longer with the last larvae taking up to 45–55 min to settle.

The decrease in settlement with time in Figure 1 is due to the fact that as the larvae settled, fewer were left in the pelagic stage. Therefore, settlement only appears to fall off. Settlement can be shown to be constant (*i.e.*, not drop off with time) in this treatment by plotting the function log (N/N − x) (where x is the number settled and N is the total number originally present in the dish; Crisp, 1974). (I preferred to present all data as % settled to emphasize the actual number of larvae settled at any given time rather than the rate of settlement with time.)

Table II

Average sulphide concentrations (measured just before each experiment began)

Treatment	Measured sulphide conc. in m*M* ($\bar{x} \pm s$)	n (# of experiments)
0.1 m*M*	0.098 ± 0.010	4
0.5 m*M*	0.508 ± 0.051	13
1.0 m*M*	1.030 ± 0.045	23
2.0 m*M*	2.040 ± 0.109	12

Table III

Decrease in sulphide after 1 h in some experiments

Treatment	Decrease in sulphide in % ($\bar{x} \pm s$)	n (# of experiments)
0.1 m*M*	17.7 ± 2.3	2
0.5 m*M*	12.9 ± 3.0	5
1.0 m*M*	14.6 ± 4.5	11
2.0 m*M*	10.2 ± 2.0	3

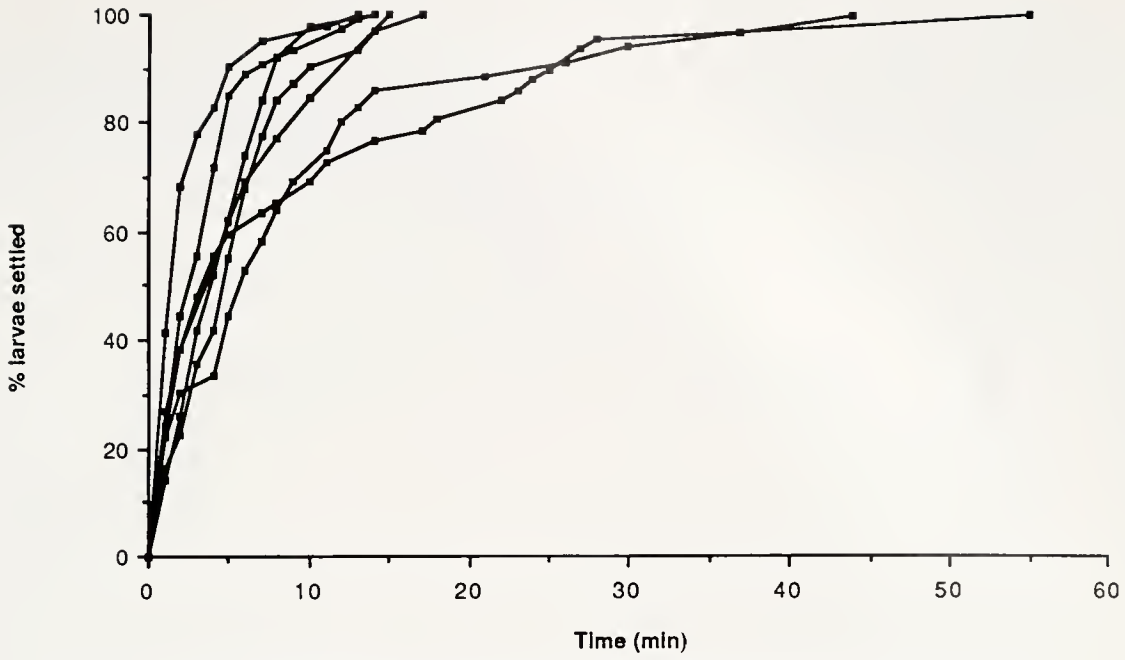

Figure 1. Settlement of freshly hatched *Capitella* sp. I larvae in sediment. Each curve represents settlement times of sibling larvae from one brood; 7 broods with between 13 and 74 larvae were tested. Settlement was observed continuously until all larvae settled.

Behavior. For these experiments the brood tubes were opened manually when the larvae began hatching (see Materials and Methods). However, there was no difference in behavior between these and 'naturally' hatched larvae. All larvae showed positive phototaxis, as described for this species by Butman *et al.* (in press). Upon leaving the brood tube, larvae immediately swam up to the surface and then toward the light sources on opposite sides of the petri dish. Larvae that settled within minutes then turned away from the light, swam down towards the mud, and crawled into it (at which point settlement time was recorded). Larvae that took longer to settle regularly swam back and forth between both light sources or clumped at the brighter light source (one ring light was inevitably a little closer than the other to the dish) before swimming down to and entering the mud. Only rarely would larvae test the mud and swim away again; larvae almost never left the mud after settling. On some occasions loss of cilia was observed within the next 5–10 min.

In several instances where brood tubes were not torn apart, I observed a few larvae that metamorphosed within the brood tube over a period of 1–2 h. The juvenile worms then left the tube and crawled into the mud.

H_2S experiments with sediment

In the presence of sediment alone behavior of older larvae (up to 35 h post-hatching) was similar to that of freshly hatched larvae, while settlement was even quicker. Fifty percent settled within 1 min, 90% within 5 min, and only 3 of 150 larvae tested took longer than 30 min to settle (Fig. 2a, Table IV a). In the presence of H_2S much longer settlement times were recorded. With increasing sulphide concentrations a higher percentage of larvae delayed settlement ($\bar{x}$ in last column in Table IV a).

Logarithmic transformation of the data for Figure 2a showed that the decrease in settlement with time in all three sulphide treatments is genuine, while settlement in the absence of H_2S does not drop off with time.

Behavior. In all treatments larvae always swam toward the light sources after introduction to the dish. In the absence of sulphide behavior was as described for freshly hatched larvae. In the presence of H_2S behavior was different depending on how quickly the larvae settled. If they settled within minutes they behaved like the freshly hatched larvae, quickly entering the mud. Some larvae that took longer to settle would repeatedly swim toward the mud, touch it with their prostomium, turn around, and swim away again (usually toward the light) until they eventually crawled into the mud; others behaved like those in the H_2S experiments without sediment and settled on the bottom of the dish away from the sediment.

H_2S and hypoxia experiments without sediment

Little or no settlement occurred in the H_2S and hypoxia controls and at the lowest (0.1 m*M*) sulphide concentration (Fig. 2b, Table IV b). Enhanced larval settlement was recorded in the 0.5, 1.0, and 2.0 m*M* sulphide

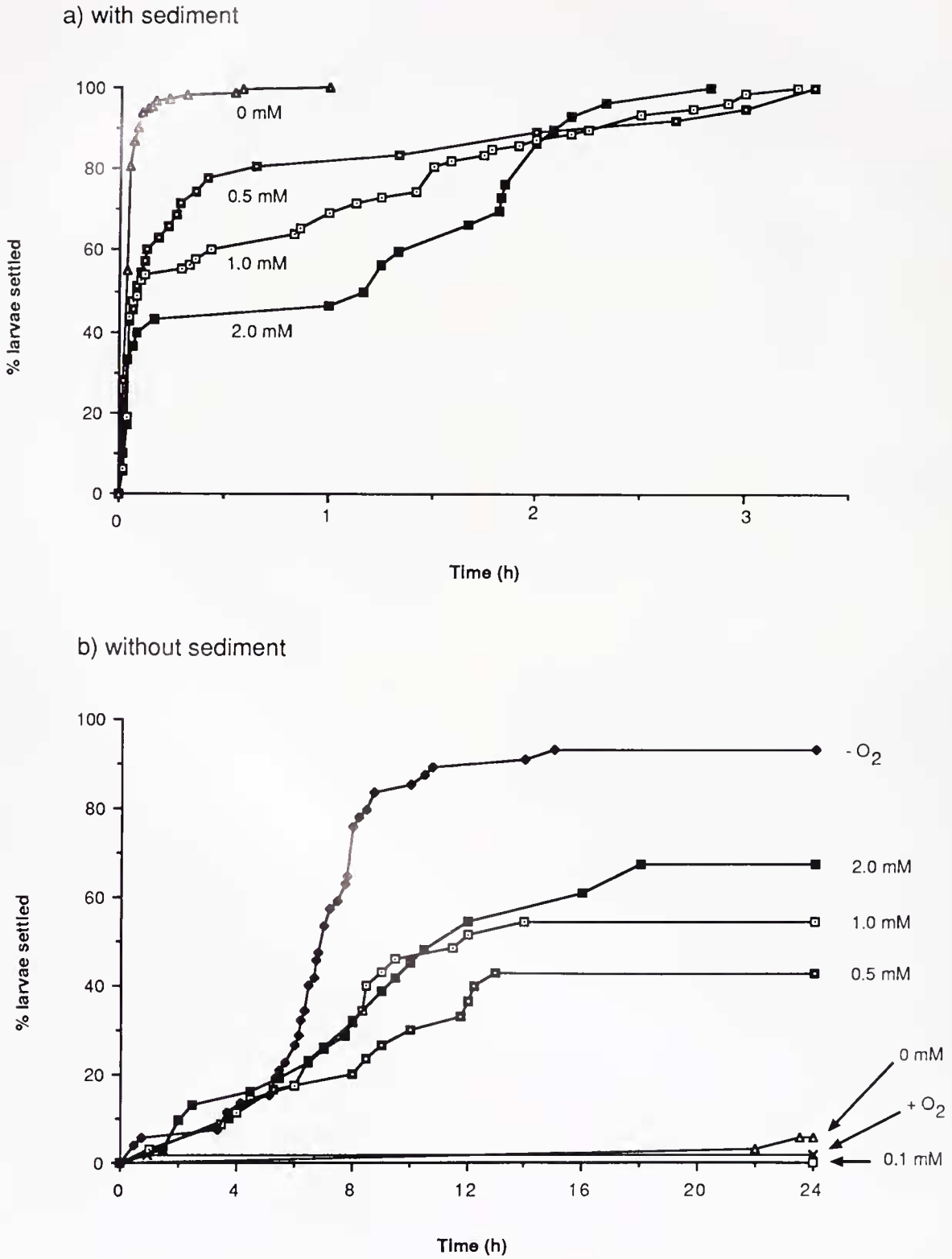

Figure 2. Settlement of *Capitella* sp. I larvae in various H_2S concentrations and hypoxia. (a) With sediment (note time scale of 3 h). Number of larvae tested: 30 (2.0 m*M*); 35 (0.5 m*M*); 80 (1.0 m*M*); 150 (0 m*M*). (b) Without sediment (note time scale of 24 h). Number of larvae tested: 20 (0.1 m*M*); 30 (0.5 and 2.0 m*M*); 35 (0 and 1.0 m*M*); 55 ($-O_2$ and $+O_2$). Each curve represents pooled data from all experiments at one treatment level. $+O_2$ = control for hypoxia treatments ($-O_2$); 0 m*M* = control for sulphide treatments (0.1, 0.5, 1.0, and 2.0 m*M*).

Table IV

Percent settlement of Capitella *sp. I larvae in various H_2S concentrations and hypoxia. (a) Percent settlement after 15 min in experiments with sediment; (b) percent settlement after 24 h in experiments without sediment. (Columns represent different treatments tested within one day on sibling larvae from one brood. Rows represent one treatment tested on different days with different broods.)*

		BROOD																		
	Treatment	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	$\bar{x} \pm s$
With sediment	(a)																				
	0 m*M*[1]	100	100	100	100	100	100	80	100	100	80	100	100	100	100	100					
							100	60			100				100						96 ± 10
								100			100										
	0.5 m*M*						0				80	100	60	40	100	80					66 ± 36
	1.0 m*M*	20	80	60	80	40	0	40		40	0	100	100	40	100	20					54 ± 34
										80		60									
	2.0 m*M*								0			40	100	0	100	20					43 ± 46
Without sediment	(b)																				
	0 m*M*[1]									0	0	0	0	0	40	0					6 ± 15
	0.1 m*M*												0	0	0	0					0 ± 0
	0.5 m*M*										100	60	0	40	60	0					43 ± 39
	1.0 m*M*									100	100	80	0	80	20	0					54 ± 46
	2.0 m*M*											80	40	60	100	100					67 ± 33
																20					
	$+O_2$[2]														0*	0	0*	0*	0*	10*	2 ± 4
	$-O_2$														100*	40	100*	100*	100*	100*	90 ± 24

* In these experiments 10 larvae, instead of 5 as in all other experiments, were used.
[1] Control for H_2S treatments.
[2] Control for hypoxia treatments.

treatments with 43, 55, and 68%, respectively, of all larvae tested settling within 24 h. However, settlement times in all three sulphide concentrations were much longer than in treatments with sediment, with less than 20% of the larvae settling within the first 6 h and less than 50% after 10 h. Higher settlement and the shortest settlement times in the absence of sediment were observed in the hypoxia experiments where 50% settled within 7 h and 90% settled within 11 h. The decrease in settlement with time (in Fig. 2b) in the three highest sulphide and hypoxia treatments is genuine.

Behavior. As in all other treatments, larvae showed a more or less pronounced phototaxis by swimming back and forth between the two light sources or clumping toward the brighter light source.

A gradual elongation of the body was visible in all larvae that eventually settled. (I was not able to distinguish if this was due to a gain in segments.) As they grew longer the larvae swam more slowly and spent more time on the bottom of the dish. They would still regularly swim up from the bottom of the dish as long as they had cilia. After the telotrochal cilia were lost, the larvae would still occasionally lift their 'heads' up from the bottom but could no longer swim. Once the prototrochal cilia were lost they remained flat on the bottom. This point was defined as settlement.

Larvae that did not settle also appeared to increase in length, but over a much longer time period (>12 h). Swimming speed decreased but not as quickly as in larvae that eventually settled.

Variation in settlement times within and among broods

Table IV shows variation in settlement times among and within broods. Low within brood variation means that either all or none of the larvae settled, as represented by 100% and 0% in the table. Among brood variation is represented by s. More than one value for a brood in a treatment means that this treatment was replicated.

In all control experiments and at the lowest sulphide concentration (0.1 m*M*) there was almost no variation in settlement times within and among broods. However, at all higher H_2S concentrations settlement time varied greatly in both cases. In comparison, the response to hypoxia was much more uniform where, with the exception of one brood, all tested larvae settled within 24 h.

Discussion

The experiments with freshly hatched larvae showed that more than 40% of the larvae tested settled almost immediately after hatching and virtually all larvae settled into the sediment in less than an hour. These results show that *Capitella* sp. I larvae are competent to respond to a settlement cue within minutes after hatching.

Somewhat older larvae (up to 35 h post-hatching) were used in all other experiments. In the presence of sediment alone, settlement behavior of older larvae did not differ from that of freshly hatched larvae. Settlement times were even shorter with 50% settling within 1 min and 96% in less than 15 min. This might be expected since it is well known that with increasing age the settle-

ment 'drive' becomes stronger in some other species of larvae (Crisp, 1974).

When 0.5 m*M*, 1.0 m*M*, and 2.0 m*M* sulphide was added to the sediment, settlement times increased up to several hours. In the absence of sediment, sulphide concentrations greater than 0.1 m*M* enhanced larval settlement, but it took up to 12 h for less than 50% of the larvae to settle.

These results question the role of H_2S as a settlement cue for *Capitella* sp. I larvae. Mud, without detectable H_2S, elicits settlement within minutes, whereas the settlement response to H_2S occurs over a time span of many hours.

The time course of Cuomo's (1985) experiments was 8 days in the settlement choice, 2 months in the microcosm, and 24 h in the *in vitro* and optimal concentration experiments. In the settlement choice and microcosm experiments sediment was used but direct observation of larval settlement was not possible. Cuomo noted that the experiments did not allow "a distinction to be made between sulphide as a potential energy source (bacterial growth as food) for juvenile *Capitella* sp. I and as a settlement cue for the larvae" (p. 174). Cuomo therefore designed the 24 h *in vitro* and optimal concentration experiments in petri dishes to better isolate and investigate the effects of sulphide. However, she did not use sediment in these experiments and thus could not observe the quick settlement response of *Capitella* sp. I larvae to mud.

The enhanced larval settlement under hypoxia and in the presence of H_2S in the experiments without sediment could be a sub-lethal toxic effect. Cuomo found that an initial concentration of 10 m*M* sulphide was lethal to most *Capitella* sp. I larvae, but at initial concentrations of 1.0 m*M* sulphide, larvae metamorphosed successfully and went on to develop and grow normally at a lower sulphide concentration (0.1 m*M* range). In this study no acute toxic effects such as arrest of ciliary movement were observed at initial concentrations up to 2.0 m*M* sulphide. However, the following factors support the toxicity hypothesis and call into question the role of sulphide as a settlement cue:

(1) The experiments without sediment by themselves might lead to the conclusion that H_2S is a settlement cue, but the larvae would be expected to show the strongest settlement response to mud plus H_2S. Instead, settlement times were prolonged up to several hours. This would be contradictory if H_2S were a settlement cue, but can be explained if the tested sulphide concentrations were sublethally toxic. With increasing sulphide concentrations a higher percentage of larvae were adversely affected and prevented from settling quickly into the mud. The behavior of larvae that settled on the bottom of the dishes away from the mud supports this hypothesis.

(2) If active habitat selection occurs in soft-bottom animals, one would expect larvae to choose an environment suitable for life. "Mechanisms must exist to ensure that larvae are brought into contact with the correct substratum, thus ensuring heaviest settlement in the most favoured substratum and least settlement in unsuitable substrata" (Gray, 1974). The strongest settlement response in the no sediment experiments was to hypoxia, but in treatments continued for up to 48 h most settled larvae died (results not shown). A similar 'response' occurred at 10 m*M* sulphide in Cuomo's experiments where highest settlement was recorded but most settled larvae died within 24 h.

One could argue that lower H_2S and higher O_2 concentrations would not be lethal. One might then expect a strong response to a lower, optimal H_2S concentration. However, in both this study and Cuomo's study, settlement *decreased* with diminishing H_2S concentrations, *i.e.*, there was no evidence for an H_2S optimum.

(3) In the sediment experiments without H_2S all larvae showed a rapid, uniform response (Table IV a) as would be expected for a settlement cue. The broad variation in the response to H_2S, not only between broods but also among siblings from the same brood (Table IV a), would mean that in the field approx. 50% of the population would not settle quickly (within minutes) in the presence of the tested sulphide concentrations. Hydrodynamic considerations (Butman, 1986) make a quick response necessary, however, and make it very unlikely that H_2S can be a cue. The broad variation in behavior is much more reminiscent of that observed in many toxicity tests, with an increasing proportion of animals adversely affected by H_2S as concentrations rise, taking longer to settle into the sediment, or failing to locate it at all.

Although care was taken to keep experimental pH fluctuations small, the standard deviation of pH values was 0.13. Since the undissolved H_2S molecule is thought to be more toxic than HS^- (National Research Council, 1979; Powell and Somero, 1986) and the degree of dissociation is mainly pH-dependent (Millero, 1986), the observed settlement variations could have been caused by differences in the concentrations of undissolved H_2S. However, correlation between various concentrations of undissolved H_2S and settlement percentages could not be proven ($R = -0.11$) in the treatment with the highest range of pH variation (1.0 m*M* sulphide with sediment: pH 7.6–8.1). Here, undissolved H_2S contents varied between 3 and 9% of the total sulphide concentration; apparently these variations were too small to affect settlement behavior.

(4) The distinction between settlement and metamorphosis is not always clear and was not defined by Cuomo (1985). Settlement in the sense of Scheltema (1974) denotes a responsive behavior and refers to the termination of pelagic existence. Metamorphosis on the other hand,

is a morphological term and is characterized by substantial morphological and physiological changes. In experiments with sediment, settlement occurred when larvae swam into the mud and stayed there; metamorphosis, defined as loss of cilia and elongation by addition of setigers (Butman *et al.*, in press), followed shortly afterwards. In experiments without sediment a behavioral response was never observed. The larvae in a slow 'metamorphosis' gradually lost their cilia, sank to the bottom of the dish, and stayed there. Metamorphosis caused loss of swimming ability—settlement as defined above never occurred.

Abnormal metamorphosis in response to sub-lethal doses of toxic substances (*e.g.*, Cu salts, ethanol, vital dyes) has been described by a number of authors (reviewed in Crisp, 1974). Although the processes involved in this abnormal metamorphosis are unclear, Crisp proposed that these substances act directly on and modify the metabolism of the larvae. H_2S, through its high affinity for cytochrome-c-oxidase (Evans, 1967; Smith *et al.*, 1977) and lack of oxygen inhibit the same metabolic pathway: aerobic respiration. The slow, abnormal metamorphosis of larvae in the H_2S and hypoxia experiments without sediment seems indicative of a modification of the larvae's metabolism whereas the quick settlement and metamorphosis in the presence of organic-rich mud is much closer to a behavioral model of stimulus and response (Burke, 1983).

Regardless of whether H_2S is considered to be a settlement cue or as here indicated sub-lethally toxic—the protracted response to H_2S over a time span of many hours is unlikely to be important for settlement in sulphide-rich sediments in the field. Although *Capitella* sp. I larvae are competent to respond to a settlement cue within minutes after hatching, their behavior (immediately swimming up away from the brood tube at hatching and toward the light) makes it likely that most larvae will spend a certain amount of time in the plankton. There, the following settlement scenario as proposed by Butman *et al.* (in press) can be suggested: selection of sites is usually not possible by horizontal swimming because water flow speeds within several mm of the seabed generally exceed swim speeds of *Capitella* sp. I larvae by factors of two to an order of magnitude (Butman, 1986; Butman *et al.*, in press). This means that while larvae can actively reject unsuitable upstream habitats, and test (and/or accept) a downstream location through vertical swimming, selection of sites is hydrodynamically constrained. Substrate-testing behavior of *Capitella* sp. I larvae can occur almost immediately after hatching, is quick, and is followed by abrupt swimming away if the substrate is rejected. Therefore the protracted response to H_2S, where hours could pass before settlement ensued, should be of no importance in active habitat selection especially when compared with a cue that elicits settlement within minutes.

The high abundances of *Capitella* in sulphide-rich sediments can not be explained by an active response of the larvae to H_2S. Since such sediments are also high in organic content, larval response to one or several cues in organic-rich mud could influence settlement at these sites. In experiments by Chesney (1985) and Chesney and Tenore (1985), the quantity of administered food (Gerber mixed cereal) significantly affected settlement rates of *Capitella* sp. I larvae, and the authors proposed that the stimulus for settlement was a "chemical cue associated with organic matter."

For effective settlement of sulphide-rich sediments, concentrations in the water column would have to be lower than 0.5 m*M* sulphide, as abnormal settlement behavior and protracted settlement times were observed at sulphide concentrations $\geq$ 0.5 m*M*. These conditions would exist over any sulphidic sediments covered by oxic water, since H_2S is oxidized quickly and the H_2S-O_2 interface is usually very narrow (Jorgensen, 1982). In the two long-term settlement experiments conducted by Cuomo (1985), exactly such conditions existed (sulphide ranged between 0.01 m*M* and 0.1 m*M*).

Thus organic-rich sediment appears to be a strong settlement cue for *Capitella* sp. I larvae. Certainly further research is needed to elucidate the effects of various physical, biological, and chemical components of organic-rich mud on active habitat selection.

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